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Phenotypic variation of *Fucus ceranoides, F. spiralis* and *F. vesiculosus* in a temperate coast (NW Portugal)

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ABSTRACT. Brown algae includes several species of *Fucus*, reported both in the tidal and intertidal zones of cold and temperate regions. Environmental parameters induce wide biological variability in intertidal algae, manifested by alterations at several levels, and this has lead to the failure of some reports to discriminate between closely related taxa, particularly *Fucus* species. As the genus *Fucus* is widely represented on the Portuguese coast, the biometric parameters of three species (*F. spiralis*, *F. vesiculosus* and *F. ceranoides*) collected from several sampling sites in Portugal, were studied over twelve months. Environmental parameters (water temperature, pH, dissolved oxygen, salinity, phosphorous - orthophosphate and total phosphate, nitrate, nitrite and ammonia) were analysed. The objective of this study was to understand how environmental parameters influence and establish morphological variation in the *Fucus* species. Canonical Correspondence Analysis (CCA), which helps define the relationships between morphological and physicochemical variables, was carried out for each species in order to determine which physicochemical parameter most affects the morphology of *Fucus*. The variable biometric that strongly separates the three *Fucus* species is the number of receptacles per thalli, and this parameter was highly correlated with *F. ceranoides*. The two others species were distinguished principally by the height of the bigger receptacle, the midrib height of the holdfast, the height of the smaller receptacle, and the midrib width of the holdfast. The CCA analysis also showed that the dominant factor influencing morphometric parameters was salinity, being always in strict correlation with water temperatures and orthophosphate. For *F. ceranoides*, physicochemical parameters (especially a higher concentration of orthophosphate and lower salinity) seem to influence morphological parameters, mainly in the raised number of receptacles per thalli. Salinity was the most important environmental parameter to distinguishing *F. spiralis* and *F. vesiculosus* in northern Portugal.

Keywords: Canonical Correspondence Analysis; Environmental parameters; *Fucus*; Morphology; Northwestern coast of Portugal.

INTRODUCTION

The genus *Fucus* is widely distributed along the Iberian Peninsula. Five species of *Fucus* have been described for Spain and four for Portugal - *Fucus spiralis* L., *F. vesiculosus* L., *F. ceranoides* L. and *F. serratus* L. (Pérez-Ruzafa et al., 1993).

Fucus shows a high level of variability in various characteristics (biological, biochemical, physiological, morphological, and life history), and these differ with geographical distribution (Kalvas and Kautsky, 1998; Pearson et al., 2000). This variation is principally related to light intensity (Major and Davison, 1998; Nygard and Ekelund, 2006), temperature (Major and Davison, 1998; Pearson et al., 2000), salinity (Ruuskanen and Bäck, 1999a b; Scott et al. 2001), coastal exposition (Ruuskanen and Bäck, 1999a; Hurd, 2000; Engelen et al., 2005), predation (Ruuskanen and Bäck, 1999a; Alstyne and Pelletreau, 2000), pH (Hurd, 2000), concentration of nutrients (Alstyne and Pelletreau, 2000; Bergström et al., 2003), hybridization (Mallet, 2005), and introgression (Coyer et al., 2006).

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The onset of reproductive periods is dependant on temperature and the day/night ratio (Hurd, 2000; Berger et al., 2001). Reproductive success depends on salinity (Andersson et al., 1994). Reproduction is not continuous, but features one or more reproductive peaks, depending on the region, and this quality also varies between species (Berger et al., 2001).

One important characteristic of the species of the genus *Fucus* is the evident zonation pattern among species. Initially, this was considered exclusively controlled by the availability of nutrients in the water (Schonbeck and Norton, 1979). However, Hurd and Dring (1990) showed that different species have different assimilation rates, suggesting other factors may be responsible for the zonation observed. Further studies showed that a complex web of physiological and biological interactions alongside the duration of periods of emersion i.e. desiccation tolerance (Beer and Kautsky, 1992) and local hydrodynamics can account for these phenomena (Hurd and Dring, 1990). According to Karez and Chapman (1998), competitive capacity is inversely related to position on the rocky substratum which can be seen in the fact that *F. vesiculosus* is more competitive than *F. spiralis,* despite the inverse localization.

For a long time, morphological variation was attributed to the high plasticity of the species in adapting to changes in environmental conditions (Chapman, 1995). However, Scott et al. (2001) showed that the expected patterns according to this hypothesis are not always observed, and several distributions can be better described as a geographic mosaic. This concept is based on the existence of stable polymorphisms (formae) within the species, which would be organized according to a patchy distribution of factors affecting the population (Scott et al., 2001). However, recent DNA sequence data and other molecular methods are beginning to show that the maintenance of morphological and genetic differences observed in *Fucus* species is paradoxical in face of potential interspecific gene flow (Billard et al., 2005). This supports the need to better understand the relationship between environmental parameters and morphologic variation, in an attempt to know even more about their biodiversity. The three taxa *F. vesiculosos* , *F. spiralis* and *F. ceranoides* are closely related, possibly as the result of a recent evolutionary radiation (Serrão et al., 1999).

In order to understand how environmental parameters influence morphological variation for each species *in situ*, we evaluated the morphological variation of the *Fucus* species *F. ceranoides*, *F. spiralis* var. *platycarpus* Batters, and *F. vesiculosus* and their relation to environmental parameters. The main environmental parameters that determine the morphological variation for each species and their geographic distribution were investigated as well. We also evaluated the mainly biometric parameters that distinguish these species. This study also investigated whether the position the algae occupy in the rocky substratum was in accordance with Karez and Chapman (1998).

MATERIALS AND METHODS

Sampling sites

Three species of *Fucus* were collected from 11 sites along the northwestern Portuguese coast, between the Minho River Estuary and Aveiro's Lagoon. These sampling sites were selected for their similarly low wave exposure,. The *Fucus* species were identified according to *Flora Phycologica Ibérica* (Martí et al., 2001) as *Fucus ceranoides*, *F. spiralis* var. *platycarpus*, and *F. vesiculosus* var. *vesiculosus*. A global positioning system (GPS, Magelan 2000XL) was used to determine sampling site coordinates (Table 1).

Table 1. Localisation and coordinates of the three species of *Fucus*.

Sampling site	*Fucus* species	Localisation	Coordinates
S1	*F. ceranoides*	Seixas-Minho river estuary	(41°53', 45" N; 8°49', 47" W)
S2	*F. ceranoides*	Caminha-Mouth of Minho river estuary	(41°52', 10" N; 8°51', 48" W)
S3	*F. spiralis* var. *platycarpus*	Carreço-coastal zone	(41°44', 63" N; 8°52', 53" W)
S4	*F. vesiculosus* var. *vesiculosus*	Areosa-coastal zone	(41°41', 71" N; 8°50', 88" W)
S5	*F. spiralis* var. *platycarpus*	Cabedelo-Mouth of Lima river estuary	(41°41', 04" N; 8°49', 88" W)
	F. vesiculosus var. *vesiculosus*		
S6	*F. spiralis* var. *platycarpus*	São Bartolomeu do Mar-coastal zone	(41°34', 50" N; 8°47', 82" W)
S7	*F. ceranoides*	Esposende-Cávado river estuary	(41°31', 85" N; 8°46', 85" W)
S8	*F. ceranoides*	Azurara-Ave river estuary	(41°24', 84"N; 8°44', 85" W)
S9	*F. spiralis* var. *platycarpus*	Cabo do Mundo-coastal zone	(41°13', 62" N; 8°42', 92" W)
S10	*F. spiralis* var. *platycarpus*	Boa Nova-coastal zone	(41°12', 23" N; 8°42', 77" W)
S11	*F. vesiculosus* var. *vesiculosus*	Barra Harbour-Aveiro Lagoon	(40°38', 69" N; 8°39', 66" W)

Over a period of twelve months *Fucus* species and superficial water samples were collected and physicochemical parameters were measured monthly in diurnal ebb low tide conditions during the crescent moon phase, between October 2001 and September 2002. In January, *Fucus* specimens at two sites (S9 and S10) could not be collected due to bad weather and sea conditions.

The geographical location of each sampling site is provided in Table 1. The most northerly site (S1) was located in Seixas, 3 km from the mouth of the Minho River Estuary. Site S2 was located in the mouth of the Minho River Estuary. Carreço (S3) was situated on the coastal zone, between Caminha and Viana do Castelo. Areosa (S4) was situated in the coast, a region very similar to S3, but rockier. Cabedelo, in the mouth of the River Lima Estuary (S5) is very close to Viana do Castelo. Site S6, on the coastal zone, was in São Bartolomeu do Mar. S7 and S8 were in the estuaries of Cávado and Ave Rivers, respectively. Site S9 (Cabo do Mundo) was located in the coastal zone, at the mouth of a small watercourse which periodically releases both domestic and industrial effluents into the sea, being located north of an oil refinery. Site S10 (Boa Nova) also located in the coastal zone, is contaminated with products from an oil refinery, from the harbour activities of the sea port of Leixões, and from marine traffic. The sampling site situated in Barra Harbour, in the Aveiro Lagoon (S11), was exposed to the type of pollution associated with a comercial sea port. Other sources of pollution in the lagoon are agricultural runoff and urban and industrial effluents (even though the main releases are now directly introduced into the sea).

Sampling Technique

At each selected sampling site, 20 specimens, *i.e.*, the minimum number recommended for a statistical analysis (Dytham, 1999), of each of the existing varieties, identified according to *Flora Phycologica Ibérica* (Martí et al., 2001) in the field, were randomly collected from a one meter square area at the lower shore level. Each specimen, always with receptacles, was carefully collected to prevent biomass loss, especially at the holdfast, which in most cases was fixed to the rocky substrata. Specimens were transported in a thermic container and were later stored at 4ºC. The measurement of morphological parameters was carried out within 48 h to minimise possible damage to biological material from predation and dehydration.

Environmental parameters

The measured physical-chemical parameters included: water temperature, pH, percentage saturation of dissolved oxygen, and salinity and these were measured *in situ*, using a Multi-Line WTW apparatus. Superficial water samples were collected monthly at each sampling site. In the laboratory, samples were filtered (0.45 µm porosity cellulose filters) and analysed immediately, for the determination of nitrate (NO_3^--N), nitrite (NO_2^--N), phosphates (orthophosphate and total phosphate), and ammonia (NH_3).

The concentration of nitrate was determined using the salicylate method according to the technique described by Rodier (1996) in a spectrophotometer JENWAY 6505 UV/VIS (Essex, United Kingdom).

Nitrite, ammonia and phosphate were analysed using a HACH DR/2000 spectrophotometer (Loveland, CO, USA) and following the Ferrous Sulphate method 8153, the Salicylate method 8155, and the Ascorbic Acid method 8048, respectively, in accordance with the Hach Water Analysis Handbook.

Morphological parameters

Sixteen biometric parameters were selected to be used as criteria for the evaluation of the influence of environmental parameters upon the studied species, according to Ruuskanem and Bäck (1999a, b). These parameters were: wet weight (WW; g), total length of the frond (TLF; cm), stipe width (SW; mm), midrib width (MW; mm) and height (MH; mm), stipe distance to the border of membrane (SDM; cm), distance of oldest dichotomy to the base (DOD; cm), midrib height (MHH) and width (MWH) of the holdfast (mm), length, width, and height of the largest (LBR, WBR and HBR, respectively) and smallest (LSR, WSR and HSR, respectively) receptacles (mm), and the number of receptacles per thalli (NREC). Monthly measurements were made using a precision balance (KERN PB, accuracy = 0.001 g), a millimetre ruler (accuracy = 0.5 mm) and a digital caliper (accuracy = 0.005 mm).

Statistical Analysis

Morphometric parameters were analysed by one-way Analysis of Variance (ANOVA), using the SigmaStat software package, version 1.00 (San Rafael, CA, USA). The Tukey multicomparison test was used to determine significant differences among the means of the *Fucus* species. Probability levels lower than 5% were considered significant ($P<0.05$). All physicochemical and morphometric data were log(10) transformed prior to the Canonical Correspondence Analysis (CCA) using a CANOCO 4.5 Statistical Package (Wales, United Kingdom) (Van den Brink and Ter Braak, 1999).

RESULTS

Environmental parameters

Seasonal and spatial variations were observed regarding the physicochemical conditions in the study area, between October 2001 and September 2002. Table 2 shows the average, maximum and minimum values of environmental parameters (water temperature, salinity, percentage saturation of dissolved oxygen, pH, orthophosphate, total phosphate, ammonia, nitrite and nitrate).

Table 2. Environmental data (water temperature, salinity, dissolved oxygen, pH, orthophosphate, total phosphate, ammonia, nitrite and nitrate), during the period of sampling.

	Water temperature (ºC)			Salinity (‰)			DO_2 (%)			pH		
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum
S1	14.78	21.90	6.50	6.6	19.7	0.2	87.8	141.1	57.0	7.39	8.14	6.18
S2	13.98	18.00	10.20	18.8	35.0	2.3	80.3	111.6	62.0	7.79	8.27	6.69
S3	15.56	19.80	11.10	32.6	37.3	28.5	125.8	190.0	67.1	8.21	8.80	7.65
S4	16.16	22.30	7.30	33.2	37.2	28.8	155.8	207.0	59.7	8.42	9.20	7.84
S5	14.99	18.50	10.70	23.3	32.2	12.1	90.2	105.2	79.0	7.88	8.29	6.93
S6	16.02	20.10	11.40	31.1	36.5	19.9	135.5	211.0	76.9	8.33	8.80	7.94
S7	16.09	23.10	9.50	4.5	16.8	0.7	90.9	148.9	65.4	7.19	8.39	6.45
S8	16.36	22.80	10.20	8.4	26.0	0.4	80.3	94.0	70.4	7.08	7.63	6.48
S9	15.45	20.50	8.60	28.5	35.7	10.5	111.7	156.0	42.5	8.11	8.81	6.92
S10	15.18	19.60	12.10	34.0	37.2	32.2	106.0	149.0	79.0	8.11	8.53	7.90
S11	16.21	22.30	8.00	27.6	37.6	11.0	82.5	107.0	61.5	7.84	8.53	7.33

	Orthophosphate - PO_4^{3-} (mg L^{-1})			Total phosphate - PO_4^{3-} (mg L^{-1})			Ammonia (mg L^{-1} NH_3)			Nitrite (mg L^{-1} NO_2^--N)			Nitrate (mg L^{-1} NO_3^--N)		
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum
S1	0.12	0.23	0.06	0.26	0.40	0.07	0.12	0.18	0.05	0.007	0.011	0.004	2.66	5.34	0.38
S2	0.08	0.14	0.04	0.25	0.43	0.07	0.06	0.15	0.01	0.005	0.008	0.003	1.11	1.98	0.38
S3	0.11	0.21	0.02	0.25	0.52	0.13	0.03	0.11	0.00	0.004	0.016	0.001	1.30	1.98	0.38
S4	0.04	0.13	0.00	0.16	0.35	0.06	0.02	0.08	0.00	0.009	0.066	0.002	2.08	9.45	0.15
S5	0.07	0.14	0.03	0.21	0.46	0.09	0.05	0.12	0.02	0.005	0.016	0.003	1.27	2.59	0.31
S6	0.08	0.18	0.03	0.18	0.31	0.06	0.03	0.11	0.01	0.011	0.054	0.004	4.08	20.74	0.54
S7	0.23	0.34	0.14	0.58	2.62	0.18	0.36	0.71	0.13	0.021	0.054	0.009	3.89	8.23	1.83
S8	1.25	1.63	0.65	1.53	2.03	0.75	0.76	1.65	0.27	0.151	0.920	0.040	4.61	7.63	1.07
S9	0.15	0.38	0.05	0.30	0.54	0.14	0.08	0.22	0.01	0.019	0.121	0.005	5.77	30.51	0.69
S10	0.06	0.12	0.00	0.31	0.92	0.07	0.06	0.20	0.01	0.006	0.015	0.003	1.44	2.29	0.76
S11	0.14	0.22	0.08	0.28	0.36	0.14	0.19	0.40	0.10	0.010	0.018	0.005	1.09	1.45	0.46

Spatial distribution of the species

Fucus species were found fixed to rocky substratum, their distribution on the Portuguese coast being apparently first determined by the presence or absence of rocky substrata. Thus, their distribution was not homogeneous in the studied area. The species *Fucus ceranoides* appeared in four stations as the only species of this genus: S1, S2, S7 and S8. The species *Fucus spiralis* var. *platycarpus* was found at S3, S6, S9 and S10, as the only species of the genus, and at S5 (in the Lima River Estuary) with *F. vesiculosus*, this last being found in the lower coastal zone. The species *Fucus vesiculosus var. vesiculosus* was also found at S4 and S11, as the only species of the genus.

Morphometric parameters of *Fucus* species

The three taxa analysed are closely related, and in order to better morphologically discriminate between these *Fucus* species, several morphometric parameters were analysed. Table 3 shows the average maximum and minimum values of sixteen biometric parameters: wet weight (WW; g), total length of the frond (TLF; cm), stipe width (SW; mm), midrib width (MW; mm), midrib height (MH; mm), stipe distance to the border of membrane (SDM; cm), distance of oldest dichotomy to the base (DOD; cm), midrib height (MHH) midrib width (MWH) of the holdfast (mm), length, width, and height of the largest (LBR, WBR and HBR, respectively) and smallest (LSR, WSR and HSR, respectively) receptacles (mm), and the number of receptacles per thalli (NREC) in each of the existing varieties. Only TLF, SW, MW, MH, WSR, HBR, HSR were significantly different for all the species analysed (P<0.05, one-way ANOVA). The SDM and WW had similar values for the species analysed. The NREC, WBR, DOD, MWH and MHH were significantly different between the *F. ceranoides* and the other two *Fucus* species analysed, and LBR and LSR were significantly different between the *F. vesiculosus* var. *vesiculosus* and the other two *Fucus* species analysed (P<0.05, one-way ANOVA).

Environmental and morphometric parameters of *Fucus* species

To evaluate the relationships between morphological and physicochemical variables, a Canonical Correspondence Analysis (CCA) was performed separately for each variety and for all physicochemical variables, but only the most relevant are presented. The advantage of the

Table 3. Morphometric data of the three species of *Fucus*.

	F. ceranoides			*F. spiralis* var. *platycarpus*			*F. vesiculosus* var. *vesiculosus*		
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum
TLF	23.51	35.82	14.18	28.16	55.56	10.13	33.54	49.57	24.45
SW	7.69	10.06	5.56	12.56	15.82	9.53	11.77	14.98	8.67
MW	1.12	1.86	0.68	1.73	2.77	1.17	1.53	2.02	0.98
MH	0.46	0.67	0.32	0.63	0.91	0.33	0.53	0.76	0.34
NREC	113.18	340.25	18.65	47.25	137.70	7.30	77.78	263.15	19.85
LBR	21.26	30.99	12.64	27.73	35.86	18.36	21.41	34.48	15.67
LSR	9.31	19.51	6.75	10.67	18.28	6.68	10.53	61.65	6.74
WBR	12.98	17.81	7.82	17.06	26.71	11.85	15.46	26.85	10.73
WSR	4.29	6.71	3.15	6.40	9.44	4.64	5.61	7.57	4.06
HBR	2.56	4.20	1.54	5.44	8.74	2.15	4.01	5.95	2.10
HSR	1.40	2.84	0.87	2.44	4.39	1.38	1.88	2.81	1.30
SDM	2.21	4.39	0.34	2.43	4.59	0.41	2.02	3.97	0.78
DOD	1.62	5.09	0.42	4.68	19.18	0.67	5.10	13.41	1.89
MWH	1.66	2.32	0.94	2.82	3.95	1.89	2.61	3.87	1.65
MHH	0.85	1.15	0.59	1.68	2.67	1.08	1.61	2.61	0.95
WW	39.73	129.78	7.64	48.78	145.67	6.42	61.05	172.10	17.97

WW: wet weight (g); TLF: total length of the frond (cm); SW: stipe width (mm); MW: midrib width (mm); MH: midrib height (mm); SDM: stipe distance to the beginning of membrane (cm); DOD: distance of oldest dichotomy (cm); MHH: midrib height of the holdfast (mm); MWH: midrib width of the holdfast (mm); LBR: length of the bigger receptacle (mm); WBR: width of the bigger receptacle (mm); HBR: height of the bigger receptacle (mm); LSR: length of the smaller receptacle (mm); WSR: width of the smaller receptacle (mm); HSR: height of the smaller receptacle (mm) and NREC: number of receptacles per thalli.

biplot is that it displays both the variables (environmental parameters as arrows) and the cases (the morphometric measurements as dots) in one plane in this multivariate analysis. The correlation of variables (arrows) to the first axis are of most importance, and cases (projected perpendicular to the arrows) closest to the tip of the arrows show the best correlation between case and parameter (Kent and Coker, 1992; Van den Brink and Ter Braak, 1999).

The first CCA (Figure 1) included all biometric parameters and the three species of *Fucus*. Axes 1 and 2 (eigenvalues, respectively, of 0.74 and 0.64; the sum of all canonical eigenvalues is 1.20) explain the 20.0% of cumulative percentage variance in species data and the 52.4% of cumulative percentage variance of the species-environment relationships; both are statistically significant (Monte Carlo Permutation Test P = 0.005). In this figure, the variable biometric that strongly separated these three species is the number of receptacles per thalli (NREC), and this is strongly correlated to *F. ceranoides*. The two others species are distinguished principally by height of the bigger receptacle (HBR), midrib height of the holdfast (MHH), height of the smaller receptacle (HSR), and midrib width of the holdfast (MWH).

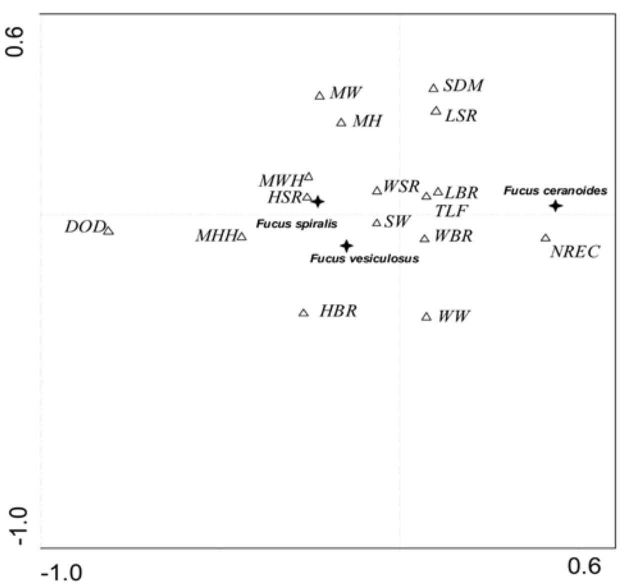
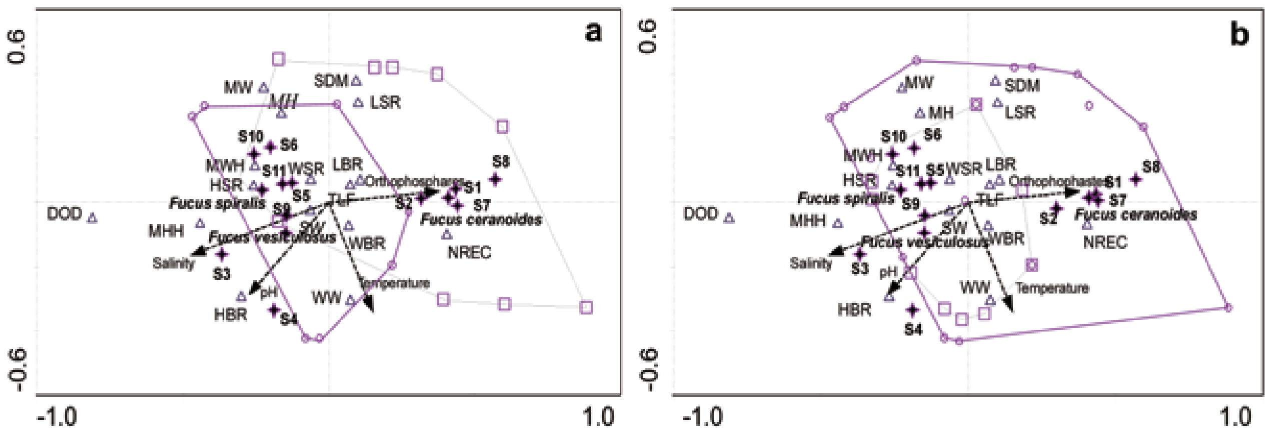


Figure 1. Correlation biplot of biometric variables based on CCA (Canonical Correspondence Analysis). WW: wet weight (g), TLF: total length of the frond (cm), SW: stipe width (mm), MW: midrib width (mm), MH: midrib height (mm), SDM: stipe distance to the beginning of membrane (cm), DOD: distance of oldest dichotomy (cm), MHH: midrib height of the holdfast (mm), MWH: midrib width of the holdfast (mm), LBR: length of the bigger receptacle (mm), WBR: width of the bigger receptacle (mm), HBR: height of the bigger receptacle (mm), LSR: length of the smaller receptacle (mm), WSR: width of the smaller receptacle (mm), HSR: height of the smaller receptacle (mm) and NREC: number of receptacles per thalli.

The Figure 2 depicts a CCA ordination diagram that shows the relationship between the biometric parameters of each species of *Fucus* and the environmental variables of the sample sites. For *Fucus ceranoides* (Figure 2a), *F. spiralis* var. *platycarpus* (Figure 2b) and *F. vesiculosus* var. *vesiculosus* (Figure 2c) two sample groups are observed between biometric parameters and environmental variables. In Figure 2a, axes 1 and 2 (eigenvalues, respectively, of 0.73 and 0.65; the sum of all canonical eigenvalues is 0.60) explain the 18.8% of cumulative percentage variance in species data and the 53.9% of cumulative percentage variance of the species-environment relationships; both are statistically significant (Monte Carlo Permutation Test P = 0.005). For *F. ceranoides* (Figure 2a) two sample groups are clearly distinguished, and one of these correlated groups between variables showed orthophosphate as the main physicochemical variable correlated to the sampling sites S1, S2, S7 and S8, where this species occurred. The second general grouping included the other physicochemical variables.

In Figure 2b, axes 1 and 2 (eigenvalues, respectively, of 0.68 and 0.33; the sum of all canonical eigenvalues is 0.30) explain the 12.5% of cumulative percentage variance in species data and the 85.4% of cumulative percentage variance of the species-environment relationships; both are statistically significant (Monte Carlo Permutation Test P = 0.005). In Figure 3c, axes 1 and 2 (eigenvalues, respectively, of 0.73 and 0.64; the sum of all canonical eigenvalues is 0.60) explain the 19.2% of cumulative percentage variance in species data and the 54.1% of cumulative percentage variance of the species-environment relationships; both are statistically significant (Monte Carlo Permutation Test P = 0.005). For *F. spiralis* (Figure 2b) and for *F. vesiculosus* (Figure 2c) two sample correlated groups between variables are not clearly delimited, but a similar pattern was observed.

Figure 3a, 3b and 3c display data obtained by CCA for different values of water temperature, salinity, and orthophosphate, respectively. In this analysis we tried to delimit several correlated groups between variables in order to conclude whether the geographic localisation of each species is determined principally by those three environmental variables. Axes 1 and 2, in Figure 3 (eigenvalues, respectively, of 0.73 and 0.64; the sum of all canonical eigenvalues is 0.60) explain the 19.2% of cumulative percentage variance in species data and the 54.1% of cumulative percentage variance of the species-environment relationships; both are statistically significant (Monte Carlo Permutation Test P = 0.005). For water temperatures (Figure 3a) two sample groups were clearly distinguished, one of these correlated groups between variables corresponded to *F. ceranoides*, and the second general grouping included *F. spiralis* and *F. vesiculosus*. For salinity (Figure 3b) three sample groups were observed, and each was associated with one species of *Fucus*, according to the gradient of this parameter. For orthophosphate (Figure 3c) two distinct sample groups



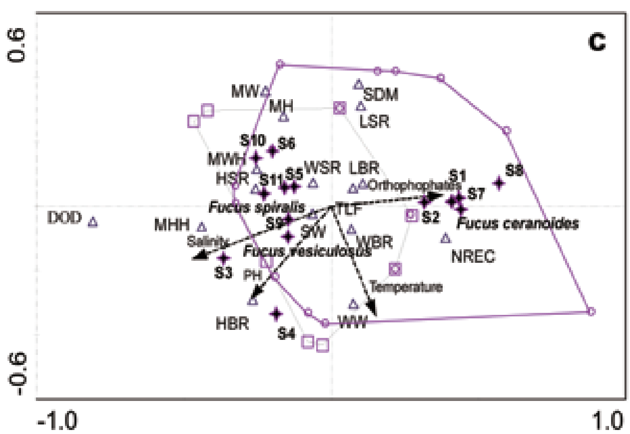


Figure 2. Correlation biplot of a) *Fucus ceranoides*, b) *F. spiralis* var. *platycarpus* and c) *F. vesiculosus* var. *vesiculosus* based on CCA (Canonical Correspondence Analysis). WW: wet weight (g), TLF: total length of the frond (cm), SW: stipe width (mm), MW: midrib width (mm), MH: midrib height (mm), SDM: stipe distance to the beginning of membrane (cm), DOD: distance of oldest dichotomy (cm), MHH: midrib height of the holdfast (mm), MWH: midrib width of the holdfast (mm), LBR: length of the bigger receptacle (mm), WBR: width of the bigger receptacle (mm), HBR: height of the bigger receptacle (mm), LSR: length of the smaller receptacle (mm), WSR: width of the smaller receptacle (mm), HSR: height of the smaller receptacle (mm) and NREC: number of receptacles per thalli.

were observed, one of these correlated groups between variables corresponded to *F. ceranoides*, and the second general grouping included *F. spiralis* and *F. vesiculosus*.

DISCUSSION

Environmental parameters

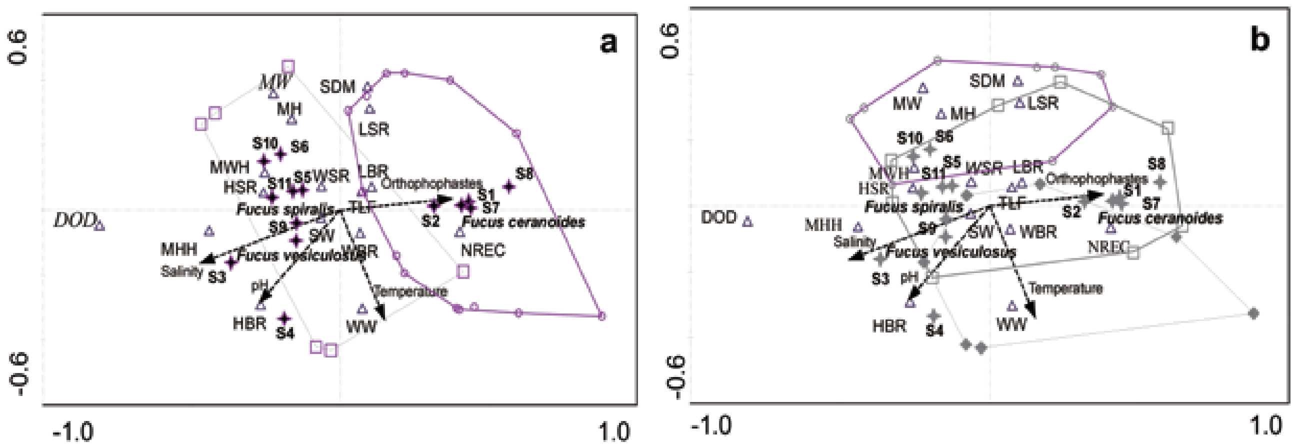
With respect to physical and chemical parameters, water temperature showed seasonal variation. Over the sampling period, mean values for water temperatures were higher at the northern sites (S1 and S2; 14.8 and 14.0ºC, respectively), and the more southerly site (S11) did not register the highest temperature (16.2ºC maximum) as expected. The highest temperature (16.4ºC) was observed at S8, possibly due to different levels of coastal exposure; the estuaries have more sheltered sites associated with the entry of warmer riverine inputs.

Surface salinity was lower at sites situated further upstream in the estuaries (S1, S7 and S8; 6.6, 4.5 and 8.4‰, respectively) and variable at the mouths of the rivers (S2 and S5). These fluctuations were mainly due to the ratio between fluvial discharge and flux, and to extention of tidal excursion (Pardal, 1998). Salinity values for coastal zones did not indicate large fluctuations, with the exception of S9 in November, where a very low value (10.5‰) was measured. This variation could be related to an increase in the flux of the stream following a period of high precipitation.

Dissolved oxygen attained its highest values at sampling site S4. The occurrence of a high biomass of green macro-algae could be, in part, responsible for these high values, as green macro-algae contribute to the increase in dissolved oxygen through their photosynthetic activity (Lyngby et al., 1999).

The pH values can vary with numerous factors, one of the most obvious being photosynthetic activity (Pardal, 1998; Hurd, 2000). Site S4 registered the highest pH value, which can be explained based on the large quantity of algae existing at this site (as testified to by the values obtained for the percentage saturation of dissolved oxygen for the same site).

Photoautotrophic organisms use nutrients in the synthesis of organic matter via the photosynthetic process; the concentration of nutrients in the medium can vary as a result of biological production (Flindt et al., 1999). A high variation in the concentration of phosphates (orthophosphate and total phosphate) was observed during the sampling period for all of the sites. This may be explained by point source or non point source discharges of urban and industrial effluents, frequent in estuarine environments and coastal zones (Pardal, 1998; Azeiteiro et al., 1999). In accordance with this, the highest mean values of orthophosphate and total phosphate were observed at S7 (0.23 and 0.58 mg/L) and S8 (1.25 and 1.53 mg/L).



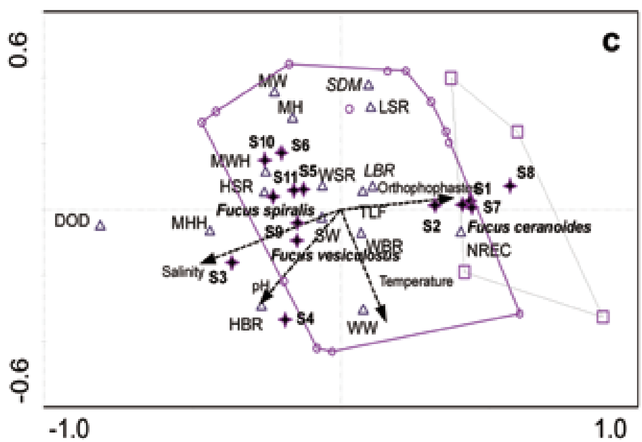


Figure 3. Correlation biplot of a) water temperatures, b) salinity and c) ortophosphates based on CCA (Canonical Correspondence Analysis. WW: wet weight (g), TLF: total length of the frond (cm), SW: stipe width (mm), MW: midrib width (mm), MH: midrib height (mm), SDM: stipe distance to the beginning of membrane (cm), DOD: distance of oldest dichotomy (cm), MHH: midrib height of the holdfast (mm), MWH: midrib width of the holdfast (mm), LBR: length of the bigger receptacle (mm), WBR: width of the bigger receptacle (mm), HBR: height of the bigger receptacle (mm), LSR: length of the smaller receptacle (mm), WSR: width of the smaller receptacle (mm), HSR: height of the smaller receptacle (mm) and NREC: number of receptacles per thalli.

The three nitrogenous components quantified in this study showed higher concentrations during autumn and the beginning of winter. These values may be due to an increase in runoff from farmlands and an increase in the release of urban and industrial effluents, principally in the estuarine environments (S7 and S8) and in Cabo do Mundo (S9), situated near a watercourse where urban effluents are released (INAG, 2000).

Morphometric parameters of *Fucus* species

The three taxa *F. ceranoides*, *F. spiralis*, and *F. vesiculosus* are closely related, possibly as the result of a recent evolutionary radiation (Serrão et al., 1999). Phylogenetic studies have used nuclear rDNA-SSU and LSU sequences to survey fucoid genera at the family level and more variable nrDNA-ITS sequences to explore relationships at the genus level (Rousseau et al., 1997; Leclerc et al., 1998; Serrão et al., 1999). However, only some of the most recent genetic analysis, like microsatellite loci, allow establishing the differences between closely related taxa of the *Fucus* species (Billard et al., 2005). The morphometric parameters used in this work allow differentiating these three closely related taxa of *Fucus* species along the Northwestern Portuguese coast, between the Minho river estuary and the Aveiro's Lagoon. The total length of the frond, stipe width, midrib width and height, length and height of the largest receptacle, and width of the smallest receptacle are significantly different for all the species analysed. The number of receptacles per thalli, width of the largest receptacle, distance of the oldest dichotomy to the base and midrib height and width of the holdfast allow us to distingush between the *F. ceranoides* and the others two *Fucus* species analysed, and the length of the largest and smallest receptacles allow us to differentiate morphologically between the *F. vesiculosus* var. *vesiculosus* and the other two *Fucus* species analysed. In contrast, several phenotypic (Burrows and Lodge, 1951; Pérez-Ruzafa et al., 1993) and genetic (Serrão et al., 1999) studies failed to differentiate between *F. ceranoides*, *F. spiralis*, and *F. vesiculosus*. However, several recent genetic studies showed a clear separation of these three *Fucus* species (Billard et al., 2005), and these species could be identified as three different genetic and morphometric species. This work also showed that *F. spiralis* is more similar to *F. vesiculosus*. This similarity was mainly due to an incomplete reproductive isolation between these two *Fucus* species (Billard et al., 2005).

Canonical Correspondence analysis also indicated that *F. ceranoides* was morphologically discriminable, principally by a raised number of receptacles per thalli. The two others species were discriminable principally by the height of the bigger receptacle, the midrib height of the holdfast, the height of the smaller receptacle, and the midrib width of the holdfast. This morphological variability is clearly evident and highlights the relevance of physiological and genetic differences. Our results indicate that in northwestern Portugal these species are principally identified visually and metrically by these five parameters.

Fucus ceranoides' physicochemical parameters (especially orthophosphate and salinity) seem to influence its morphological parameters, mainly in the raised number of receptacles per thalli. For *F. ceranoides* (Figure 2c) two sample groups are clearly distinguishable, and this grouping shows a correlation between the raised concentration of orthophosphate and the sampling sites S1, S2, S7 and S8, where this species occurred and the salinity was lower. The correlation between orthophosphate and *F. ceranoides* was also observed in Figure 3c, where two sample groups were also observed, according to the concentration of orthophosphate. Kraufvelin et al. (2006) observed that nutrient enrichment may have adverse or beneficial effects on reproduction, in rocky shore communities, and these effects were mainly dependent on the species involved. Our results showed that the reproduction of *F. ceranoides* may also be affected by the higher environmental concentrations of orthophosphate, observed at the sampling sites S1, S2, S7 and S8, where this species occurred. Another possible explanation is that low salinity areas are affected by freshwater runoff bringing sewage, inducing higher environmental concentrations of orthophosphate. Our results showed that *F. ceranoides* always has more receptacles than the other two *Fucus* species since they have more dichotomies and thus more reproductive tips per thalli, and *F. ceranoides* occurred only at low salinity sites. The effect of low salinity on reproduction is corroborated by Ruuskanen and Bäck (1999a) whose studies in the Gulf of Finland suggest that salinity is the main environmental factor behind seasonal variations in reproduction.

A similar pattern was seen in *F. spiralis* var. *platycarpus* and in *F. vesiculosus*, but the two sample groupings are not clearly delimited, indicating that all the physicochemical parameters influence morphological parameters.

Regarding the remaining Figure 3a, 3b and 3c (water temperature, salinity and orthophosphate, respectively) a consistent pattern seems to emerge. *Fucus ceranoides* is present at sites with higher concentrations of orthophosphate and lower salinity while the opposite was true for the other two *Fucus* species. Geographic localisation of this species is determined principally by three environmental variables. However, salinity is the main parameter distinguishing *F. spiralis* from *F. vesiculosus* in northern Portugal.

The vertical distribution of the species in the study area agrees with the description in the available literature, *F. spiralis* var. *platycarpus* is the variety which inhabits the upper eulittoral zone, and *F. vesiculosus* is observed in the lower eulittoral zone (Hurd and Dring, 1990; Karez and Chapman, 1998; Cairrão et al., 2004).

In conclusion, our results clearly indicate that the dominant fact influencing morphometric parameters is salinity, being always in strict correlation with water temperatures and orthophosphate. In addition, for all species of *Fucus* studied, morphological variability is clearly evident, and several biometric parameters analysed seem to establish the differences between these closely related taxa of the *Fucus* species.

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LITERATURE CITED

Alstyne, Van K.L. and K.N. Pelletreau. 2000. Effects of nutrient enrichment on growth and phlorotannin production in *Fucus gardneri* embryos. Mar. Ecol. Prog. Ser. **206:** 33-43.

Andersson, S., L. Kautsky, and A. Kalvas. 1994. Circadian and Lunar gamete release in *Fucus vesiculosus* in the atidal Baltic Sea. MEPS **110:** 195-201.

Azeiteiro, M., J.C. Marques, and P. Ré. 1999. Zooplankton Annual cycle in the Mondego river estuary (Portugal). Arquivos do Museu Bocage. Vol. III, **8:** 239-264.

Beer, S. and L. Kautsky. 1992. The recovery of net photosynthesis during rehydration of three *Fucus* species from the Swedish west coast following exposure to air. Botanica Marina **35:** 487-491.

Berger, R., T. Malm, and L. Kautsky. 2001. Two reproductive strategies in Baltic *Fucus vesiculosus* (Phaeophycea). Eur. J. Phycol. **36:** 265-273.

Bergström, L., L. Berger, and L. Kautsky. 2003. Negative direct effects of nutrient enrichment on the establishment of *Fucus* vesiculosus in the Baltic Sea. Eur. J. Phycol. **38:** 41-46.

Billard, E., C. Daguin, G. Pearson, E. Serrão, C. Engel, and M. Valero. 2005. Genetic isolation between three closely related taxa: *Fucus vesiculosus*, *F. spiralis* and *F. ceranoides* (Phaophyceae). J. Phycol. **41:** 900-905.

Burrows, E.M. and S.M. Lodge. 1951. Autecology and species problem in *Fucus*. J. Mar. Biol. Assoc. UK **30:** 161-175.

Cairrão, E., M. Couderchet, A.M.V.M. Soares, and L. Guilhermino. 2004. Glutathione-S-transferase activity of *Fucus* spp. as a biomarker of environmental contamination. Aquat. Toxicol. **70:** 277-286.

Chapman, A.R.O. 1995. Functional ecology of fucoid algae: twenty-three years of progress. Phycologia **34:** 1-13.

Coyer, J.A., G. Hoarau, M.P. Oudot-Le, W.T. Stam, and J.L. Olsen. 2006. A mtDNA-based phylogeny of the brown algal genus *Fucus* (Heterokontophyta; Phaeophyta). Mol. Phylog. Evol. **39:** 209-222.

Dytham, C. 1999. Choosing and Using Statistics: A Biologists Guide. Blackwell Science, Oxford.

Engelen, A.H., P. Aberg, J. Olsen, W.T. Stam, and A. Breeman. 2005. Effects of wave exposure and depth on biomass, density and fertility of the fucoid seaweed *Sargassum polyceratium* (Phaeophyta, Sargassaceae). Eur. J. Phycol. **40(2):** 149-158

Flindt, M.R., M.A. Pardal, A.I. Lillebø, I. Martins, and J.C. Marques. 1999. Nutrient cycling and plant dynamics in estuaries: A brief review. Acta Oecol. **20:** 237-248.

Hurd, C.L. 2000. Water motion, marine macroalgal physiology, and production. J. Phycol. **36:** 453-472.

Hurd, C.L. and M.J. Dring. 1990. Phosphate uptake by intertidal algae in relation to zonation and season. Mar. Biol. **107:** 281-289.

INAG. 2000. Planos das bacias Hidrográficas dos rios Luso – Espanhóis – Síntese. Caracterização e Diagnostico. Instituto da Água, Direcção de serviços de recursos Hídricos, Divisão de recursos Subterrâneos.

Kalvas, A. and L. Kautsky. 1998. Morphological variation in *Fucus vesiculosus* populations along temperature and salinity gradients in Iceland. Mar. Biol. Assoc. UK **78:** 985-1001.

Karez, R. and A.R.O. Chapman. 1998. A competitive hierarchy model integrating roles of physiological competence and competitive ability does not provide a mechanistic explanation for the zonation of three intertidal *Fucus* species in Europe. OIKOS **81:** 471-494.

Kent, M. and P. Coker. 1992. Vegetation description and analysis: a practical approach. Wiley, New York.

Kraufvelin, P., M.E. Frithjof, C. Hartvig, and T.L. Bokn. 2006. Nutrient addition to experimental rocky shore communities revisited: delayed responses, rapid recovery. Ecosystems **9:** 10076-1093.

Leclerc, M.C., B. Véronique, L. Guillaume, and B. De Reviers. 1998. Low divergence in rDNA ITS sequences among five species of *Fucus* (Phaeophyceae) suggests a very recent radiation. J. Mol. Evol. **46:** 115-120.

Lyngby, J.E., S. Mortensen, and N. Ahrensberg. 1999. Bioassessment techniques for monitoring of eutrophication and nutrient limitation in coastal ecosystems. Mar. Pollt. Bull. **36:** 212-223.

Major, K.M. and I.R. Davison. 1998. Influence of temperature and light on growth and photosynthetic physiology of *Fucus evanescens* (Phaeophyta) embryos. Eur. J. Phycol. **33:** 129-138.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. **20(5):** 229-237.

Martí, M.C.B., T.G. García, A.G. Garreta, I. Pérez-Ruzafa, M.A.R. Siguan, and J.R. Lluch. 2001. Flora Phycologica Iberica. Vol. I. *Fucales. In* A.G. Garreta (ed.), Universidad de Murcia, Murcia.

Nygard, C.A. and N.G.A. Ekelund. 2006. Photosynthesis and UV-B tolerance of the marine alga *Fucus vesiculosus* at different sea water salinities. J. Appl. Phycol. **18:** 461-467.

Pardal, M.A.C. 1998. Impacto da Eutrofização nas Comunidades Macrobentónicas do braço Sul do Estuário do Mondego (Portugal). Ph.D. Thesis, Universidade de Coimbra, Coimbra.

Pearson, G.A., L. Kautsky, and E. Serrão. 2000. Recent evolution in Baltic *Fucus vesiculosus*: reduced tolerance to emersion stress compared to intertidal (North Sea) populations. Mar. Ecol. Prog. Ser. **202:** 67-79.

Pérez-Ruzafa, I., T. Gallardo, and R. Gómez-Cancio. 1993. Numerical taxonomy of taxa of the genus *Fucus* in the Iberian Peninsula. Hydrobiologia **260/261:** 81-90.

Rodier, J. 1996. L'analyse de l'eau: eaux naturelles, eaux résiduaires, eaux de mer. 8 Ed. Dunod, Paris.

Rousseau, F., M.C. Leclerc, and B. de Reviers. 1997. Molecular phylogeny of Fucales (Phaeophyceae) based on partial large-subunit rDNA sequence comparisons. Phycol. **6:** 436-438.

Ruuskanen, A. and S. Bäck. 1999a. Morphological variation of northern Baltic Sea *Fucus vesiculosus* L. Ophelia **50:** 43-59.

Ruuskanen, A. and S. Bäck. 1999b. Does environmental stress affect fertility and frond regeneration of *Fucus vesiculosus*? Ann. Bot. Fennici. **36:** 285-290.

Schonbeck, M.W. and T.A. Norton. 1979. The effects of brief periodic submergence on intertidal fucoid algae. Estuar. Cstl Mar. Sci. **8:** 205-211.

Scott, G.W., S.L. Hull, S.E. Hornby, F.G. Hardy, and N.J.P. Owens. 2001. Phenotypic variation in *Fucus spiralis* (Phaeophycea): morphology, chemical Phenotype and their relationship to the environment. J. Phycol. **36**: 43-50.

Serrão, E., L.A. Alice, and S.H. Brawley. 1999. Evolution of the Fucaceae (Phaeophyceae) inferred from nrDNA-ITS. J. Phycol. **35:** 382-94.

Van den Brink, P.J. and C.J.F. Ter Braak. 1999. Principal response curves: Analysis of time dependent multivariate responses of biological community to stress. Environ. Toxicol Chem. **18:** 138-148.

生長於溫帶沿岸之 *Fucus ceranoides*, *F. spiralis* 及 *F. vesiculosus* 的表現型差異

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褐藻包含 *Fucus* 屬之若干種已知在寒帶、溫帶之潮汐區及潮間帶。環境之變數導致潮間帶海藻之極大生物變異性，反映在幾個層次，因此使得某些科研工作無法區分密切相關之類別（主要是 *Fucus* 屬下之品種）。因為 *Fucus* 屬廣存於葡萄牙海岸，我們在 12 個月之間分別在不同採樣點收集三個品種 ：*Fucus ceranoides*, *F. spiralis* 及 *F. vesiculosus* 分別研究其生物參數。分析之環境參數有 ： 水溫，酸碱度，溶氧量，鹽分，含磷量（正磷酸及全磷量），硝酸，亞硝酸及氨。本研究之目的在於 ： 嘗試瞭解環境因子變數如何影響及建立 *Fucus* 品種之形態變異。每一品種的形態及物理化學變數兩者間之相關以 Canonical Correspondence Analysis (CCA) 分析之，以便找出影響 *Fucus* 品種之形態的主要物理化學變數。強烈區分 *Fucus* 三品種之生物變數為 ： 葉狀體之葉托數目 ； 而此參數和 *F. ceranoides* 高度地相關。其他兩品種則主要根據 ： 較大葉托之高度，夾器之中脈高度，較小葉托之高度，及夾器之中脈寬度。CCA 分析也顯示 ： 影響形態變數之主宰因子為鹽份（總是和水溫及正磷酸量二者密切相關）。以 *F. ceranoides* 而言，物理化學變數（尤其是高濃度之正磷酸鹽和低鹽份）似乎影響諸形態變數，尤其是增加每一葉狀體之葉托數目。鹽份乃區分葡萄牙北方之 *F. spiralis* 及 *F. vesiculosus* 最重要之環境因子。

關鍵詞 ：Canonical Correspondence Analysis (CCA)；環境因子 ；*Fucus*；形態 ； 葡萄牙北海岸。